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SCIENTIFIC RESEARCH ON PAPAYER SPECIES AS SOURCES OF CODEINE, MORPHINE AND THEBAIN^{1/}

No. 37

SIMULTANEOUS IDENTIFICATION OF MAJOR ALKALOIDS IN POPPY PLANTS
BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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^{1/} This ST/SCA/SER.J/... series of documents was previously entitled "Scientific research on Papaver bracteatum".

General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

INTRODUCTION

Several chromatographic methods have been reported for quantitative analysis of alkaloids in poppy plants or gum opium using either the isocratic or the gradient elution techniques (1-15). The use of an isocratic method is not practical due to the fact that one has to use two or more different solvent mixtures as the mobile phase for the complete analysis. Also, in this method the chromatographic column has to be cleaned or regenerated after several runs in order to obtain reproducible results.

The objective of this work is to present a rapid and sensitive method by which the major alkaloids present can be analyzed in one run, and therefore routine and automated repetitive analyses are possible.

METHOD

1. Extraction

0.2-0.5 g samples ground to Tyler -48 mesh size were weighed and placed in a 20 ml volumetric flask. 10 ml of 4% H_2SO_4 solution were added together with 2-3 mg brucine (dihydrate) which served as the internal standard. The contents of the flask were shaken for one hour. A clear extract was obtained by centrifugation and placed in a 50 ml separatory funnel. An equal volume of hexane was added to the filtrate, the mixture was shaken and the organic phase was discarded. 25% ammonia solution was added to the aqueous phase to adjust the pH to 10-11. Alkaloids were then extracted with an equal volume of organic phase consisting of chloroform and iso-propanol (3:1).

2. Apparatus

A Waters Assoc. Model ALC/GPC 204 liquid chromatograph equipped with a second Model 6000A solvent delivery system. Model 660 programmer and Model U6K universal injector were used in these studies. μ Bondapak C_{18} (4mm ID X 30 cm) column was selected because it provided high column efficiency and excellent reproducibility. Alkaloids were determined at 280 nm using a UV detector.

3. Reagents

All reagents were obtained from Merck A.G. and were of analytical grade. Alkaloid standards were obtained from T.M.O. (The Turkish Soil Products Office) and Knoll A.G.

4. Solvent systems

- (a) Polar solvent: 1% (w/v) $(\text{NH}_4)_2\text{CO}_3$ solution in water;
(b) Less polar solvent: methanol.

5. Conditions

Gradient elution from 55% to 100% methanol in 15 minutes by curve No. 9. Initial conditions were attained in 5 minutes by reversing the programme using curve No. 6 after each analysis. The flow rate of the mobile phase was kept constant at 2 ml/min.

6. Calculations

Brucine (dihydrate) was used as the internal standard. K values for the alkaloids calculated from equation [1] are given in Table I with their respective standard deviations.

$$K = \frac{W}{W_B} \cdot \frac{A_B}{A} \quad [1]$$

where:

- $\underline{W}$ = Weight of an alkaloid in standard solution,
 $\underline{W_B}$ = Weight of brucine (dihydrate) in standard solution,
 $\underline{A}$ = Peak area of an alkaloid,
 $\underline{A_B}$ = Peak area of brucine (dihydrate).

The alkaloid content of an unknown sample was calculated according to equation [2] given below:

$$\underline{W} (\%) = K \frac{A}{A_B} \frac{W_B}{W_S} \cdot 100 \quad [2]$$

where:

- $\underline{W_S}$ = weight of the unknown sample.

TABLE I

K values of alkaloids with respect to brucine (dihydrate)

<u>Compound</u>	<u>K (as in equation [1])*</u>
Morphine	3.75 ± 0.07
Codeine	3.78 ± 0.05
Thebaine	0.72 ± 0.02

* Valid only with the chromatographic conditions mentioned above.

RESULTS AND DISCUSSION

The results of the method developed are in a good agreement with the results for reference samples provided by the U.N. Narcotics Laboratory (Table II).

TABLE II
Results of analysis of several poppy samples

Sample	Extraction method	% Morphine	% Codeine	% Thebaine
UN Sample I	%5 HOAc	1,71*	0,065	-
UN Sample I	%2 H ₂ SO ₄	1,73*	-	-
UN Sample I	%4 H ₂ SO ₄	1,77	0,014	0,010
Turkish sample Iznik	%4 H ₂ SO ₄	0,52	-	-

* Private communication - U.N. Narcotics Laboratory, Geneva.

The analysis of the same poppy samples was repeated by thin-layer and gas chromatographic techniques in our laboratory. We believe that the HPLC method proves to be more reliable and less time consuming than the above mentioned methods. Among the various extraction methods described in the literature, we found that 4% H₂SO₄ led to the highest alkaloid content. In order to avoid time loss required for the complete filtration of the mixture, brucine is added into the extraction mixture and the first 5 ml of the filtrate analyzed. Any organic moiety, other than the alkaloids, which may contaminate the HPLC columns is removed by hexane. Brucine with a similar pK_a value as morphine (16) is assumed to be fully protonated in the presence of 4% H₂SO₄ and hence remains in the aqueous phase.

The K values were calculated both using the peak heights and areas. The latter gave much less deviation.

The claimed decomposition of thebaine in acidic medium was checked (17) and no such decomposition was observed in 4% H₂SO₄ for one hour.

The chromatogramme of the test mixture, morphine, codeine and thebaine with brucine (dihydrate) is shown in Figure 1. The chromatogrammes of the U.N. poppy sample and the Turkish poppy sample are given in Figure 2 and Figure 3, respectively.

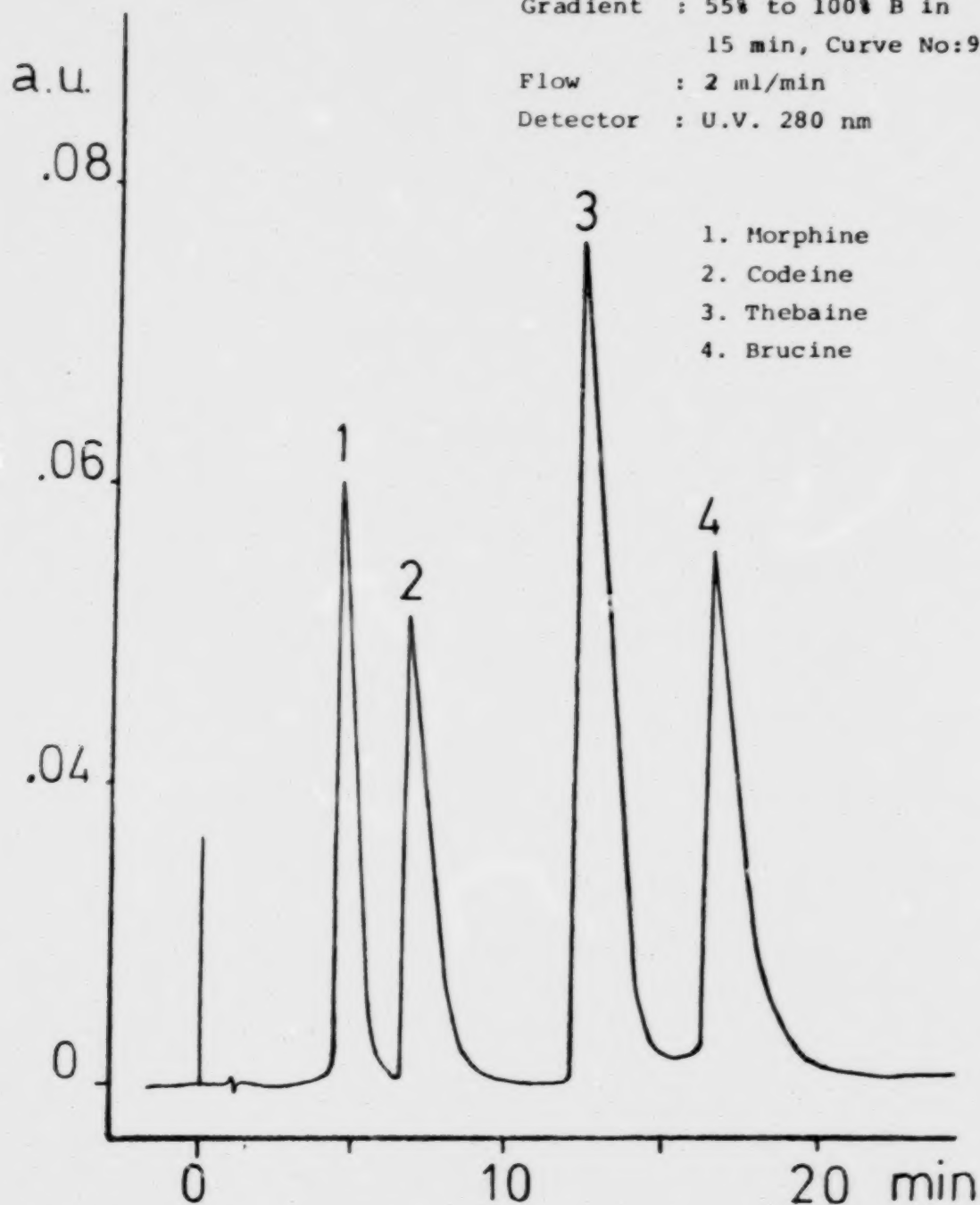
ACKNOWLEDGEMENTS

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Packing : μ Bondapak C₁₈
Solvent : A:1% (NH₄)₂CO₃ soln.
B:Methanol
Gradient : 55% to 100% B in
15 min, Curve No:9
Flow : 2 ml/min
Detector : U.V. 280 nm



- 1. Morphine
- 2. Codeine
- 3. Thebaine
- 4. Brucine

Figure 1 - Chromatogramme of the test sample.

Packing : μ Bondapak C_{18}
Solvent : A:1% $(NH_4)_2CO_3$ soln.
 B:Methanol
Gradient : 55% to 100% B in
 15 min, Curve No:9
Flow : 2 ml/min
Detector : U.V. 280 nm

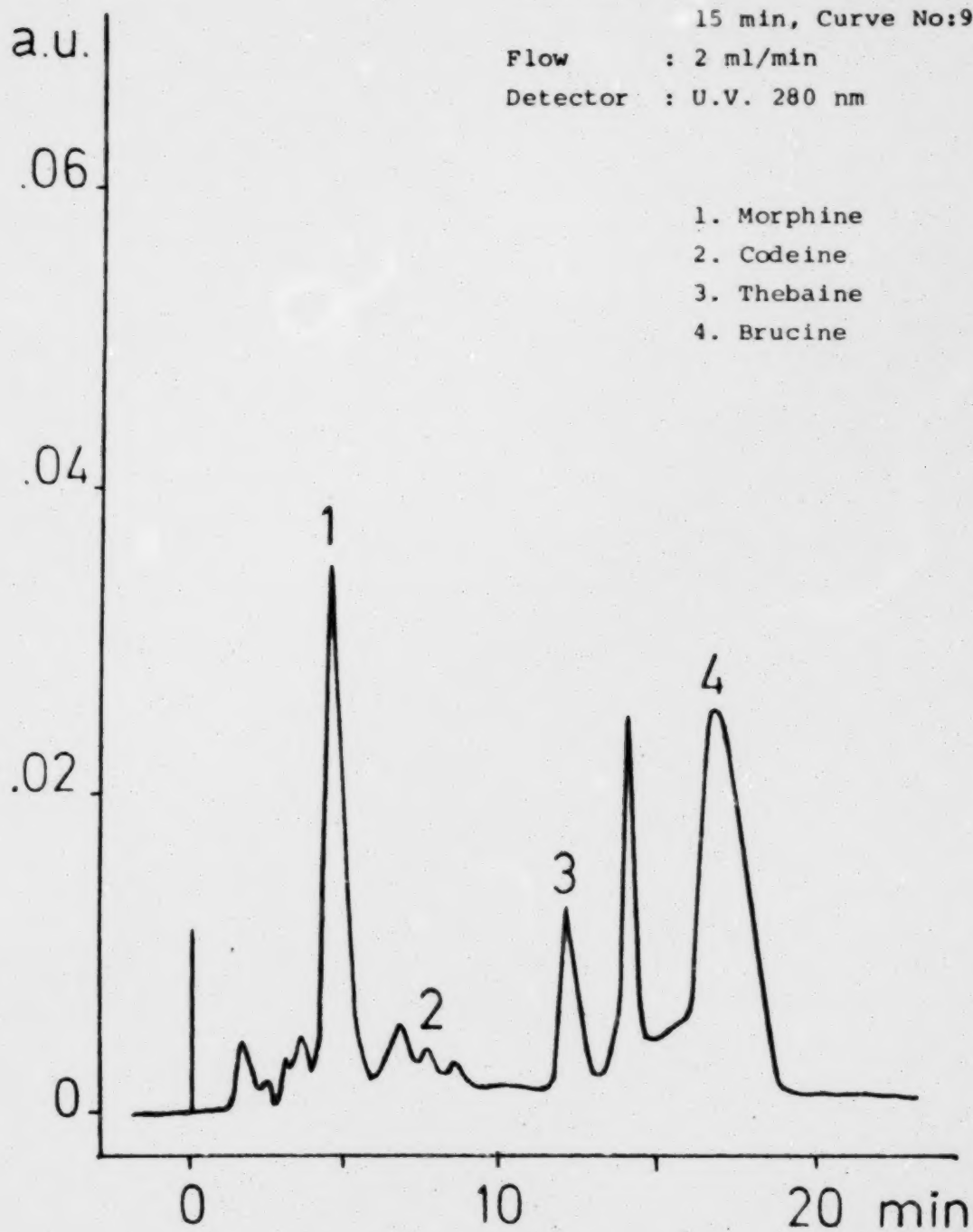


Figure 2 - Chromatogramme of UN sample I.

Packing : Bondapak C₁₈
Solvent : A: 1% (NH₄)₂CO₃ soln.
B: Methanol
Gradient : 55% to 100 % B in
15 min, Curve No.9
Flow : 2 ml/min
Detector : U.V. 280 nm

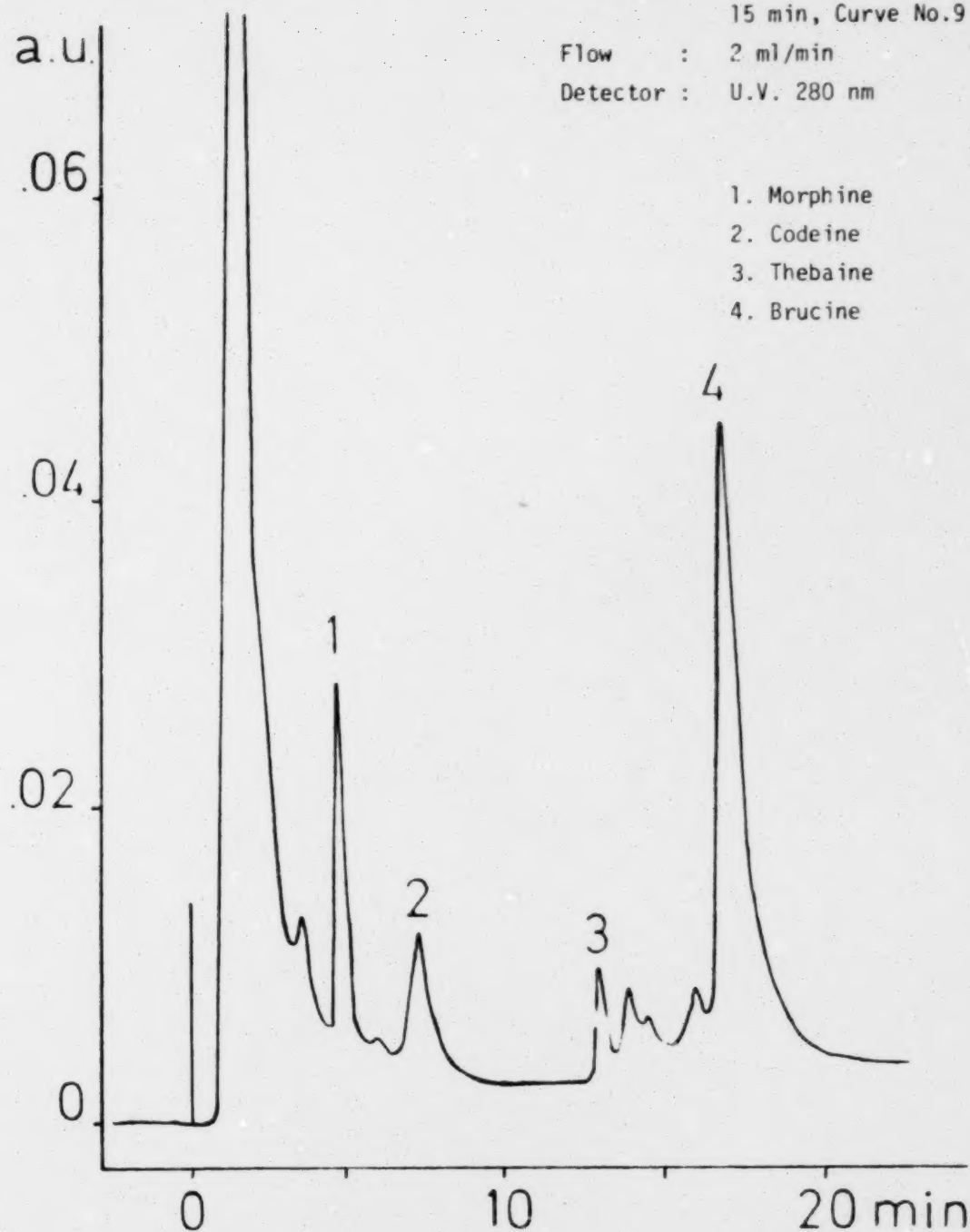


Figure 3 - Chromatogramme of Turkish poppy sample.